

Appl. No. 09/857,433  
Amendment dated: April 20, 2005  
Reply to OA of: October 20, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

Claims 1-23(canceled).

24(new). In a method for assaying homocysteine concentration in a biological fluid sample, said method comprising the steps of;

- i) contacting said biological fluid sample with homocysteine desulphurase (HDS) whereby to generate alpha-ketobutyrate,
- ii) generating a signal corresponding to said alpha-ketobutyrate,
- iii) subsequently assessing the thus-generated signal and
- iv) relating the assessed signal to the homocysteine concentration in said biological fluid sample;

wherein the improvement comprises enhancing the signal to noise ratio by means of contacting said biological fluid with a reducing agent, prior to step i), subsequently with HDS, in step i), and with an agent which binds, oxidises or renders inactive said reducing agent after step i) and before step ii).

25(new). In a method for assaying homocysteine concentration in a biological fluid sample, said method comprising the steps of;

- i) adding an aqueous liquid to a lyophilisate comprising HDS and at least one cryoprotectant or lyoprotectant, whereby to provide a liquid reagent and
- ii) contacting said biological fluid sample with said liquid reagent, whereby to form at least one homocysteine conversion product,
- iii) generating a signal corresponding to said least one homocysteine conversion product,

iv) subsequently assessing the thus-generated signal and relating the assessed signal to the homocysteine concentration in said biological fluid sample;  
wherein the improvement comprises forming said lyophilisate in the substantial absence of any thiol-containing cryoprotectants or lyoprotectants.

26(new). The method as claimed in claim 25 wherein said liquid reagent is an aqueous liquid containing homocysteine desulfurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer.

27(new). In a method for assaying homocysteine concentration in a biological fluid sample, said method comprising the steps of;

i) contacting said biological fluid sample with HDS, whereby to form alpha-ketobutyrate,  
ii) generating a signal corresponding to said alpha-ketobutyrate,  
iii) subsequently assessing the thus-generated signal and relating the assessed signal to the homocysteine concentration in said biological fluid sample;

wherein the improvement comprises having enhancing the signal to noise ratio by means of treating said biological fluid sample with an agent which serves to deactivate pyruvates prior to step i).

28(new). In a method for assaying homocysteine concentration in a biological fluid sample, said method comprising the steps of;

i) contacting said biological fluid sample with and immobilised HDS, whereby to form at least one homocysteine conversion product,  
ii) generating a signal corresponding to said least one homocysteine conversion product,

iii) subsequently assessing the thus-generated signal and relating the assessed signal to the homocysteine concentration in said biological fluid sample;

wherein the improvement comprises in step i);

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- ia) contacting said biological fluid sample with and immobilised HDS under conditions whereby said homocysteine binds to said HDS but does not react, whereby to form bound homocysteine,
- ib) separating said bound homocysteine from the remainder of said biological sample,
- ic) treating said bound homocysteine whereby to form at least one homocysteine conversion product.

29(new). An assay for homocysteine concentration in a biological fluid sample, said method comprising the steps of;

- i) contacting said biological fluid sample with HDS, whereby to form alpha-ketobutyrate,
- ii) generating a signal corresponding to said alpha-ketobutyrate,
- iii) subsequently assessing the thus-generated signal and relating the assessed signal to the homocysteine concentration in said biological fluid,

wherein the improvement comprises enhancing the signal to noise ratio by means of filtering said biological fluid through a size exclusion filter and centrifuging, whereby to remove pyruvates, prior to step i).

30(new). The method as claimed in claim 24, said method further comprising at least one method selected from;

adding an aqueous liquid to a lyophilisate comprising HDS and at least one cryoprotectant or lyoprotectant, whereby to provide a liquid reagent and contacting said biological fluid sample with said liquid reagent, wherein said lyophilisate is formed in the substantial absence of any thiol-containing cryoprotectants or lyoprotectants;

treating said biological fluid sample with an agent which serves to deactivate pyruvates before contacting with said HDS;

contacting said biological fluid sample with and immobilised HDS under conditions whereby said homocysteine binds to said HDS but does not react, whereby

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to form bound homocysteine and separating said bound homocysteine from the remainder of said biological sample; and

filtering said biological fluid sample through a size exclusion filter and centrifuging, whereby to remove pyruvates, before contacting said biological fluid sample with said HDS.

31(new). The method as claimed in claim 25, said method further comprising at least one method selected from the group consisting of;

treating said biological fluid sample with an agent which serves to deactivate pyruvates before contacting with said HDS;

contacting said biological fluid sample with and immobilised HDS under conditions whereby said homocysteine binds to said HDS but does not react, whereby to form bound homocysteine and separating said bound homocysteine from the remainder of said biological sample; and

filtering said biological fluid sample through a size exclusion filter and centrifuging, whereby to remove pyruvates, before contacting said biological fluid sample with said HDS.

32(new). The method, as claimed in claim 27, said method further comprising at least one method selected from the group consisting of;

contacting said biological fluid sample with and immobilised HDS under conditions whereby said homocysteine binds to said HDS but does not react, whereby to form bound homocysteine and separating said bound homocysteine from the remainder of said biological sample; and

filtering said biological fluid sample through a size exclusion filter and centrifuging, whereby to remove pyruvates, before contacting said biological fluid sample with said HDS.

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33(new). The method as claimed in claim 30 wherein said method comprises;  
contacting said biological fluid with a reducing agent, subsequently with HDS and  
with an agent which binds, oxidizes or renders inactive said reducing agent after being  
contacted with said HDS and before generating said signal corresponding to said  
alpha-ketobutyrate;

adding an aqueous liquid to a lyophilisate comprising HDS and at least one  
cryoprotectant or lyoprotectant, whereby to provide a liquid reagent and contacting said  
biological fluid sample with said liquid reagent, wherein said lyophilisate is formed in the  
substantial absence of any thiol-containing cryoprotectants or lyoprotectants; and

treating said biological fluid sample with an agent which serves to deactivate  
pyruvates before contacting with said HDS.

34(new). The method as claimed in claim 33 wherein said liquid reagent is an  
aqueous liquid containing homocysteine desulfurase, a thiol-reducing reagent, and a  
proteinaceous or non-proteinaceous stabilizer.

35(new). The method as claimed in claim 27 wherein the agent which serves to  
deactivate pyruvates is hydrogen peroxide.

36(new). The method as claimed in claim 35 wherein the hydrogen peroxide is  
neutralized with catalase prior to contacting the sample with said homocysteine  
converting enzyme.

37(new). The method as claimed in claim 27 wherein after the sample is treated  
with the agent, the sample is heated at 40-60 C for 15 to 60 minutes prior to contacting  
with said homocysteine converting enzyme.

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38(new). The method as claimed in claim 27 wherein the agent which serves to deactivate pyruvates is selected from the group consisting of pyruvate carboxylase, pyruvate oxidase, and lactate dehydrogenase.

39(new). The method as claimed in claim 29 wherein the sample is filtered with a 30 kD exclusion filter.

40(new). The method as claimed in claim 25 wherein a NAD<sup>+</sup>/NADH cycling reaction is used to generate a colored compound the concentration of which may be correlated to the concentration of homocysteine in the biological fluid sample.

41(new). The method as claimed in claim 27 wherein a NAD<sup>+</sup>/NADH cycling reaction is used to generate a colored compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

42(new). The method as claimed in claim 28 wherein a NAD<sup>+</sup>/NADH cycling reaction is used to generate a colored compound the concentration of which may be correlated to the concentration of homocysteine in the biological fluid sample.

43(new). The method as claimed in claim 29 wherein a NAD<sup>+</sup>/NADH cycling reaction is used to generate a colored compound the concentration of which may be correlated to the concentration of homocysteine in the biological fluid sample.